PATENT SPECIFICATION

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COMPLETE SPECIFICATION

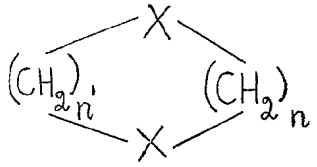
Oxygenated Cycloalkanes and the Preparation Thereof

We, THE UPJOHN COMPANY, a corporation organized and existing under the laws of the State of Delaware, United States of America, of 301 Henrietta Street, Kalamazoo, State of Michigan, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a novel method for the introduction of oxygen into a cycloalkanol molecule, to certain novel products produced by the process of this invention, and to certain novel derivatives thereof.

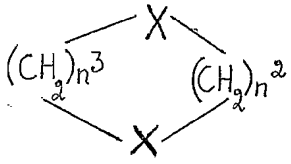
More particularly this invention relates to the introduction of oxygen into a cycloalkanol containing from 11 to 14 carbon atoms, inclusive, by subjecting the cycloalkanol to oxygenating strains of microorganisms.

The novel process of the present invention is applicable to compounds represented by the general formula:



wherein X in each case is $>C=O$ or $>C<\begin{matrix}OH\\H\end{matrix}$, and may be the same or different, and wherein n is a whole number from 5 to 9, inclusive, n′ is a whole number from 3 to 5, inclusive, and in which the sum of $n+n'+2$ is not less than 11 and not more than 14.

Some of the novel compounds which are produced according to the present invention are dioxygenated cyclododecanes of the general formula:—



... A

[Pr

where n^2 is a whole number from 5 to 7 inclusive and n^3 is a whole number from 3 to 5 inclusive in which the sum of n^2+n^3+2 is 12 and wherein X is in each case $>C=O$ or $>C<^{OH}_{H}$ and may be the same or different.

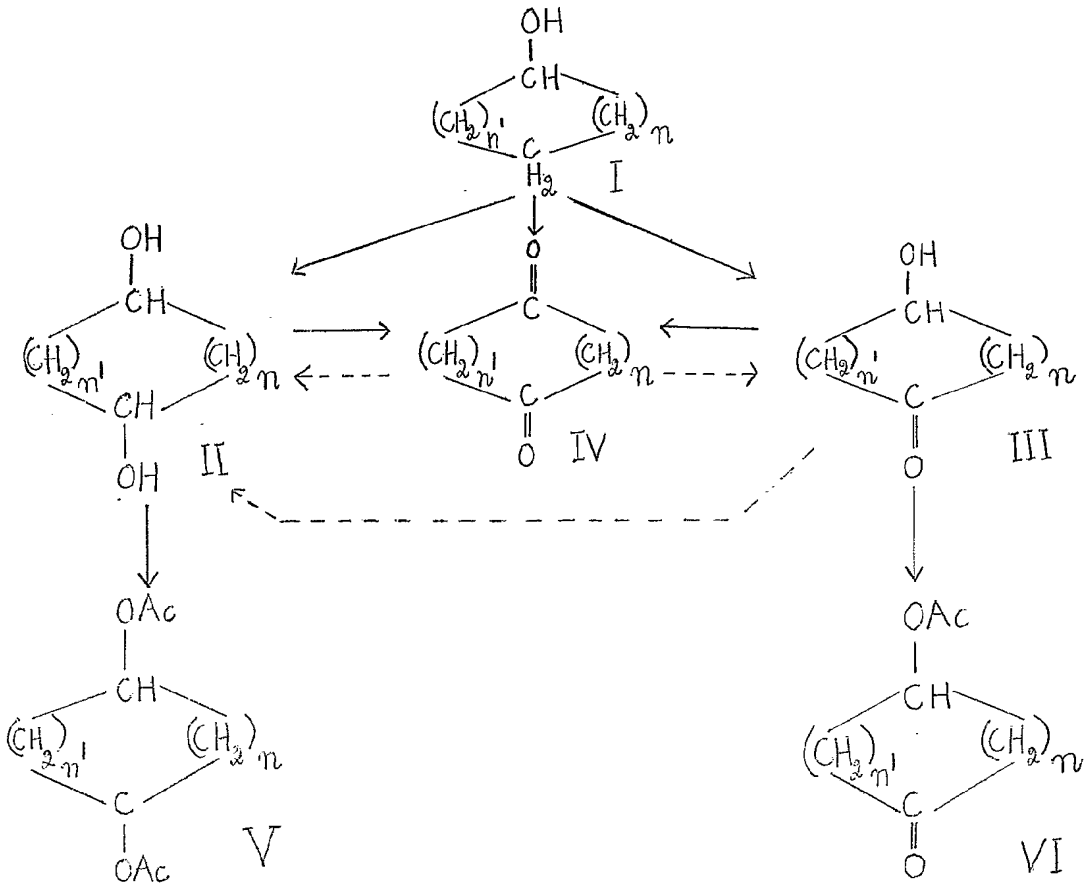
Other novel compounds produced are:—
cyclotridecane-1,5-diol;
cyclotridecane-1,6-diol;
cyclotridecane-1,7-diol;
cyclotetradecane-1,5-diol;
cyclotetradecane-1,6-diol;
cyclotetradecane-1,7-diol;
5-hydroxycyclotridecanone;
6-hydroxycyclotridecanone;
7-hydroxycyclotridecanone;
5-hydroxycyclotetradecanone;
6-hydroxycyclotetradecanone;
and 7-hydroxycyclotetradecanone.

All the above novel compounds are central nervous system depressants are are useful as sedatives and general anesthetics in mammals, particularly in humans and animals. For example, they can be used as sedatives and anesthetics in the laboratory manipulation of experimental animals such as mice and rats.

The said novel compounds can be prepared and administered in a wide variety of oral or parenteral dosage forms singly, or in admixture with other active compounds. They can be associated with a pharmaceutical carrier which can be solid material such as lactose, starch or chalk, or a liquid such as water or aqueous alcohol, in which the compound is dissolved, dispersed, or suspended. The solid compositions can take the form of tablets, powders, capsules or pills, preferably in unit dosage forms for simple administration of precise dosages. The liquid compositions can take the form of solutions, emulsions, suspensions, syrups or elixirs.

In addition to having therapeutic activity, the compounds of Formula A are useful as perfumery agents and for the preparation of perfumery agents, being closely related to certain musk principles. The compounds of Formula A are also useful as plasticisers for cellulosic and vinyl polymers, and as intermediates in chemical synthesis. For use as plasticisers the compounds of Formula A can be used singly or in mixtures of the position isomers, for example, the mixture obtained from the bioconversion step can be purified as by crystallization and used directly as a plasticiser.

The products and process of this invention are represented by the following reaction scheme which also illustrates the preparation of the esters of the novel compounds of the invention:—



wherein n and n′ have the meanings previously given and Ac is the acyl radical of an organic carboxylic acid, preferably a hydrocarbon carboxylic acid containing from 1 to 12 carbon atoms, inclusive.

The microbiological process of this invention comprises subjecting a cycloalkanol containing from 11 to 14 carbon atoms, inclusive, in the ring structure (I) to the action of an oxygenating strain of fungus to produce a mixture of the corresponding dioxygenated compounds of Formulae II, III and IV. The operational conditions, reaction procedure and details are those known in the art of bioconversion as illustrated in steroid bioconversion, e.g., in British Patent Specification No. 724,094. In the bioconversion of the compounds of Formula I, oxygenation is effected at the 5, 6 and 7-positions of the ring. The mono- and dihydroxy compounds of Formulae II and III can also be oxidized chemically to produce the corresponding diketo compounds IV. The mono- and diketo compound of Formulae III and IV can be reduced to give the corresponding dihydroxy compound II, or the compounds of Formula IV can be partially reduced to give the corresponding hydroxy ketones of Formula III. The hydroxy groups of the compounds of Formula II and III can be acylated if desired to give the corresponding monoacyloxy- and diacyloxy compounds of Formulae V and VI, respectively. The above processes are hereinafter described in detail.

The starting materials for the process of this invention are cycloundecanol, cyclododecanol, cyclotridecanol and cyclotetradecanol, all of which are known in the art.

Fungi of the subphylum Eumycetes are those used in the practice of this invention. Eumycetes include the classes Phycomycetes, Ascomycetes, Basidiomycetes and Fungi Imperfecti (Deuteromycetes).

Orders of the above classes of fungi which are especially useful in the practice of this invention and representative genera thereof are listed as follows:

Mucorales: Absidia, Blakeslea, Circinella, Chaetocladium, Cunninghamella, Helicostylum, Gongronella, Mucor, Parasitella, Phycomyces, Rhizopus.
Endomycetales: Ascocybe, Byssochlamys, Cephaloascus, Endomyces, Petasospora, Endomycopsis.
Taphrinales: Protomyces, Taphridium, Taphrina.
Eurotiales: Ctenomyces, Carpenteles, Eidamella, Emericillopis, Eurotium, Microascus, Penicilliopsis, Talaromyces, Thielava.
Myringiales: Elsinoe, Dothiora.
Dothideales: Acrosperum, Capnodium, Chaetothyrum, Cymadothea, Dangeardiella, Dothidea, Rhopographus, Scorias.
Hemisphaeriales: Schizothyrina, Schizothyrium.
Hysteriales: Farlowiella, Gloniella, Gloniopsis, Glonium, Hysterium, Lophium, Mytilidion, Ostreion.
Hypocreales: Calonectria, Calostilbe, Claviceps, Cordyceps, Creonectria, Epichloe, Gibberella, Hypocrea, Loramyces, Melanospora, Nectria, Nectriella, Neocosmospora, Ophionectria, Sphaerostilbe, Subbaromyces.
Sphaeriales: Adelopus, Chaetomium, Chaetomidium, Clathrospora, Didymella, Endothia, Glomerella, Guignardia, Mycrosphaerella, Physalospora, Xylaria, etc.
Phacidiales: Coccopeziza, Colpoma, Clithris, Phacidiella, Phacidium, Sphaerothyrium.
Helotiales: Allophylarea, Pezezella, Corynella, Dermea, Godronia, etc.
Pezizales: Ascobolus, Discomycetella, Morchela, Patella, Pyronema, Sowerbyella, Wolfina, etc.
Ustilaginales: Bryophytomyces, Cintractia, Cintodia, Entyloma, Farysia, Graphiola, Schizonella, Sorosporium, Tilletia, Tolyposporium, Urocystis, Ustilago.
Tremellales: Auricularia, Ceratobasidium, Calocera, Dacromyces, Helicobasidium.
Agaricales: Aleurodiscus, Alnicola, Boletus, Clavaria, Coprinus, Clitocybe, Collypia, Coniophora, Corticum, Deconia, Entaloma, Fomes, Hygrophorus, Lentinellus, Lentinus, Panacolus, Paxillus, Penicophora, Pholiota, Pleurotus, Plicatura, Polyporus, Poria, Psalliota, Scizophyllum, Sparrassis, Stereum, Tricholoma, Trametes.
Phallales: Mutinus, Phallus, Simblum.
Lycoperdales: Bovista, Calvatia, Greastrum, Lycoperdon.
Sclerodermatales: Gastrosporium, Lycogalopsis, Phellorinia, Sphaerobolus, Tulostoma.
Nidulariales: Crucibulum, Cyathus, Nidula.
Sphaeropsidales: Ascochyta, Coniothyrium, Dendrophroma, Diplodia, Diplodina, Polyopeus, Sphaeropsis.
Melanconiales: Actinonema, Colletotrichum, Cryptosporium, Entomosporium, Melanconium, Myxosporium, Pestalotia, Septomyxa, Steganosporium, Tuberculariella.
Moniliales: Acremonium, Aspergillus, Botrytis, Curvularia, Cylindrocarpon, Dactylium, Gliocladium, Helminthosporium, Mycelia, Penicillium, Sepedonium, Sporotrichum, Tricothecium.
Mycelia Sterilia: Microxyphium, Papulospora, Rhizoctonia, Sclerotium.

Typical species of the above listed genera are listed in the table in Example 1. These fungal organisms including their mutants can be obtained from known sources, such as the Northern Utilization Research and Development Branch, U.S. Department of Agriculture, Peoria, Illinois, (N.R.R.L.), the American Type Culture Collection (A.T.C.C.), Washington, D.C., or Centraalbureau voor Schimmelcultures (C.B.S.), Baarn, Holland.

In the practice of this invention, the bioconversion can be effected in a growing or resting culture of the microorganisms or by spores or washed cells of the microorganism. The selected cycloalkanol (I) can be added to the culture during the incubation period, or the cycloalkanol can be included in the nutrient medium prior to inoculation. Assimilable sources of nitrogen and carbon should also be present in the culture medium. Also an adequate sterile air supply should be maintained during the conversion, for example, by the conventional techniques of exposing a large surface of the medium or by passing air through a submerged culture.

Sources of nitrogenous growth promoting factors are those normally employed in such processes. They may be natural organics such as corn steep liquor, soy bean meal, yeast extracts, peptone and/or distillers solubles, or synthetics such as nitrates and ammonium compounds.

Suitable energy source materials which can be utilized in the process of this invention include meat extracts and peptone which also serve as nitrogen sources or other conventional carbon containing materials such as glycerol or carbohydrates of the type of glucose, fructose, dextrose, sucrose, lactose, maltose, dextrines, starches and whey. These materials may be used either in a purified state or as concentrates such as whey concentrate, corn steep liquor, grain mashes and the like, or as mixtures of the above. The cycloalkanol can be added to the culture during the incubation period or included in the medium prior to sterilization or inoculation. The preferred, but not limiting range of concentration of the cycloalkanol in the culture is about 0.1 to 0.5 grams per liter. The time interval required for the action of the enzymatic system of the micro-organisms employed may vary considerably, the range of about 2 to 120 hours being practical but not limiting; 72 hours being generally satisfactory. The process of the bioconversion and the completion of the bioconversion reaction is conveniently determined by paper strip chromatography, vapor phase chromatography or thin film chromatography [Textbook, Chromatography, Heftman (1961) Reinholt Publishing Co., New York, N.Y.]. The temperature need be maintained only within such range as supports life, active growth, or the enzyme activity of the fungus. A temperature between about 25 to about 32 degrees centigrade is preferred. The medium can desirably have a pH before inoculation of between about 4 to 8 although a higher or lower pH can be used. A pH of about 4 to 6 is preferred for growth of the fungus.

After completion of the fermentation, the resulting dioxygenated cycloalkanes (II, III and IV) are recovered from the fermentation reaction mixture by conventional methods. For example, the fermentation reaction mixture, including the fermentation liquor and mycelia, can be extracted with a nonpolar, water-immiscible organic solvent, e.g., methylene chloride, chloroform, carbon tetrachloride, ethylene chloride, trichloroethylene, ether, amyl acetate or benzene.

The fermentation liquor and mycelia can be separated by conventional methods, e.g., filtration and then separately extracted with suitable solvents. The mycelia can be extracted with either water-miscible or water-immiscible solvents, e.g., acetone or alcohol, or the mycelia can be merely washed with water, in cases where little or no product is contained in the mycelium, and the wash water added to the beer filtrate. The fermentation liquor, freed of mycelia, can then be extracted with water-immiscible solvents, e.g., the solvents listed above. The extracts are combined, dried, as for example over anhydrous sodium sulphate, and the solvent removed by conventional methods, e.g., evaporation or distillation. The dioxygenated cycloalkanols (II, III and IV), thus obtained, can be further purified by conventional methods, e.g., recrystallization or chromatography.

Isolation of the position isomers of compounds (II) (III) and (IV), obtained as a product of the fermentation can be accomplished by means of chromatography and fractional crystallization, if desired. However, a much more convenient and advantageous method is first to oxidize under acidic, neutral or slightly basic conditions the crude dioxygenated cycloalkanols (II and III) as they occur in the bioconversion product mixture, in accordance with methods known in the art for oxidizing secondary hydroxy groups to ketones, for example, Fieser and Fieser, 3rd Ed., pages 127—129, 193 and 194, Rienhold Publishing Corp., New York, N.Y.). Thus, the crude bioconversion product containing the oxygenated cycloalkanols can be dissolved in an inert organic solvent, e.g., acetone, benzene, methylene chloride, or a tertiary alcohol, e.g., t-butanol, and then oxidized with aqueous chromic acid, e.g., Jones reagent, potassium permanganate, or t-butylhypochlorite in t-butanol, to produce the corresponding cycloalkanones (IV) which can then be separated by chromatography, crystallization or both to obtain the corresponding position isomers (IV), namely, the corresponding cycloalkan-1,5-dione, cyclo-alkan-1,6-dione and cycloalkan-1,7-dione, in purified form.

The compounds of Formula IV, thus obtained, can then be reduced, preferably under neutral or acidic conditions, in accordance with methods known in the art for reducing carbonyl groups, to produce the corresponding dihydroxy compounds (II) and the corresponding monohydroxy compounds (III). For example, reduction may be effected with two molar equivalents or more of, for example, hydrogen in presence of a catalyst such as palladium, platinum or Raney nickel under neutral conditions; sodium in an alkanol, or with a reducing agent such as, for example, lithium aluminum hydride, sodium borohydride, primary isobutyl magnesium bromide, or lithium tri-tertiary butoxy aluminum hydride, to produce the dihydroxy compound (II). Reaction of the compounds of Formula IV with one molar equivalent of the above named reducing agents is productive primarily of the monohydroxy compounds (III), plus

some of the corresponding dihydroxy compounds (II). These monohydroxy and dihydroxy compounds can be separated from each other by conventional methods, e.g., chromatography and/or crystallization. Primary isobutyl magnesium bromide and lithium tritertiary butoxy aluminum hydride are preferred reducing agents in the preparation of the monohydroxy compounds.

The compounds of Formula II and III can be acylated to give the corresponding diacyloxy and monoacyloxy compounds (V and VI), respetcively, in accordance with methods known in the art for acylating secondary hydroxy groups, for example, by reaction with the appropriate acid anhydride or acid halide, ester by ester exchange, or acid in the presence of an esterification catalyst.

Suitable acylating agents are organic carboxylic acids, particularly hydrocarbon carboxylic acids containing from one to twelve carbon atoms, inclusive, or the acid anhydrides or acid halides thereof. For example, an aliphatic acid, formic, acetic, propionic, butyric, valeric, hexanoic, lauric, trimethylacetic, isobutyric, isovaleric, tertiary butylacetic, a cycloaliphatic acid, e.g., β - cyclopentylpropionic, cyclohexanecarboxylic, cyclohexylacetic, an alkaryl acid, e.g., benzoic, phenylacetic, β-phenylpropionic, *o*-, *m*-, *p*-toluic, a saturated dibasic acid (which can be converted into water soluble, e.g., sodium, salts), e.g., succinic, adipic, a monobasic unsaturated acid, e.g., acrylic, crotonic, undecylenic, propiolic, 2-butynoic, undecolic, cinnamic, dibasic unsaturated acids, (which can be converted into water soluble, e.g., sodium, salts), e.g., maleic and citraconic, or the acid anhydrides and acid halides thereof. If the acylating agent is the free acid, the reaction is preferably effected in the presence of an esterification catalyst, for example, *p*-toluenesulphonyl chloride, trifluoroacetic anhydride, *p*-toluenesulphonic acid, trifluoroacetic acid or sulphuric acid.

The process of this invention is applicable generally throughout the field of Eumycetes. The following examples are intended to illustrate the process applied to representative and typical individual organisms. The following examples are for the purpose of illustrating the best mode contemplated of carrying out the invention and to supplement the foregoing disclosure of the invention with additional descriptions of the manner and process of carrying out the invention so as further to enable workers skilled in the art to do so. It is possible that in the field of Eumycetes there may be isolated individual organisms that do not behave as described, and it is to be understood that these are relatively few, and are exceptions to the behaviour of the Eumycetes as a group. The examples supplementing the foregoing disclosure include representative individual organisms of the orders Mucorales, Endomycetales, Hypocreales, Sphaerales, Pharcidiales, Helotiales, Pezizales, Ustilaginales, Agaricales, Tycoperdales, Sclerodermatales, Nidulariales, Melanconiales, Moniliaes, and Mycelia Sterilia.

In British Patent Specification No. 918,444 there is described and claimed a cyclododecanediol containing non-vicinal hydroxyl groups of melting point 99—100°C. and yielding on reaction with phenyl isocyanate, a phenylurethan of melting point 210—211°C. when prepared by subjecting 1,2: 5,6 - diepoxycyclododeca - 9 - ene to catalytic hydrogenation at a temperature of 50—150°C. under superatmospheric pressure.

In British Patent Specification No. 803,178 there is described and claimed a process for the reduction of compounds which contain multiple bonds between carbon and oxygen and/or between carbon and nitrogen wherein there is used as reducing agent a dialkyl aluminium hydride or an aluminium triisoalkyl or a complex compound of one of these compounds with an alkali metal hydride and such reduction products when so produced.

EXAMPLE 1.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 100 ml. of this sterilized medium was inoculated with a 72-hour vegetative growth of one of the organisms listed in the following table, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 to 48 hours, or when a moderate to heavy growth of mycelium was apparent by visual observation, of agitation, a solution of 20 mg. of cyclododecanol in 1 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium (the whole culture) was extracted 4 times with a volume of methylene chloride equal to about one-fourth the volume of the whole culture. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue. The residue thus obtained was assayed

by paper strip chromatography and found to consist of mixtures of dioxygenated cyclododecanes in the several oxidation states, namely,

cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone, and
7-hydroxycyclododecanone.

The paper strip chromatography was carried out using the Bush B—3 system, in which the sheet is equilibrated overnight at 34°C. in the vapor from a mixed solvent composed of 6667 ml. of Skellysolve C hexanes, 333 ml. of benzene, 800 ml. of methanol and 200 ml. of water, and developed with the nonpolar phase. The diones and hydroxy ketones are detected by spraying the developed sheet with 2,4 - dinitrophenylhydrazine reagent, and the diols and hydroxyketones are detected by dipping the developed sheet in phosphomolybdic reagent [L. M. Reineke, Anal. Chem. *28*, 1853—58 (1952)].

The crude residue in each of the experiments was dissolved in 1 ml. of acetone and oxidized at room temperature by the addition of a visible excess of Jones chromic acid reagent. The excess oxidant was destroyed by the addition of a few drops of isopropyl alcohol and the mixture evaporated to dryness. Water (2 ml.) was added, and the products were extracted with 2 ml. of methylene chloride. The extract was evaporated to dryness and the residue subjected to paper chromatography by the method described above and to gas-liquid (vapor phase) chromatography. Cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione were identified as constituents of the extract.

The gas-liquid chromatography was carried out by injecting 0.02 ml. of a 1:1 ethylene dichloride-methanol solution containing 1 mg. of sample into an injection port heated at 263°C. The column was packed with 5% of a methyl silicone polymer (General Electric SE·30) on 30—60 mesh fluorinated polymer (Haloport F). The temperature was programmed at 6.4°C./minute from 90°C. to 275°C. at a helium flow rate of 35—40 ml. per minute. The detection block temperature was 265°C.

The above experiments were repeated substituting in place of the corn steep liquor medium 100 ml. of a medium prepared of 50 g. of dried malt extract and 5 g. of commercial peptone diluted to 1 liter, the normal pH was about 6.5. Essentially identical results were obtained differing only in the relative amounts of each of the position isomeric cyclododecanediones formed.

The following microorganisms can be used in place of those tabulated below.

Mucor microsporous, ATCC 8541
Rhizopus nigricans, ATCC 6227b
Curvularia pallescens, NRRL 2381
Aspergillus ochraceus, NRRL 405—260—4718
Aspergillus niger, ATCC 8740
Aspergillus candidus, ATCC 1002
Aspergillus oryzae, ATCC 10196
Aspergillus wentii, ATCC 10583
Penicillium camemberti, ATCC 6985
Penicillium brevi-compactum, ATCC 9056
Penicillium citrinum, ATCC 10105
Penicillium claviformae, ATCC 10426
Penicillium decumbens, ATCC 10436
Penicillium griseo-fulvum, ATCC 11885
Penicillium ochraceum, ATCC 10474
Pencillium rugulosum, ATCC 10128.

Table of Microorganisms

Absidia glauca, ATCC 7852a, 7852b
Cunninghamella blakesleeana, ATCC 8688a
Mucor griseocyanus, ATCC 1207a
Rhizopus arrhizus, ATCC 11145
Byssochlamys fulva, CBS

Cephaloascus subcordata, CBS
Calonectria decora, CBS
Claviceps purpurea, CBS
Cordyceps miliaria, CBS
Creonectria rubricarpa, ATCC 9551
Giberella saubinettii, CBS
Hypocrea rosellus, CBS
Loramyces junicola, CBS
Nectria cosmariospora, CBS
Adelopus balsamicola, CBS
Chaetomidium barbatum, CBS
Clathrospora perminada, CBS
Didymella applanata, CBS
Endothia parasitica, CBS
Glomerella fusariodes, CBS
Glomerella lycopersici, CBS
Guignardia bidwellii, ATCC 9559
Mycosphaerella ligulicola, CBS
Physalospora tucumanensis, CBS
Xylaria vaporaria, CBS
Clithris quercina, CBS
Allophylarea lythri, ATCC 6492
Dermea bicolor, CBS
Dermea libocedri, CBS
Morchella esculenta, CBS
Patella abundens, CBS
Pyronema confluens, CBS
Cintractia sorghi, CBS
Aleurodiscus amorphus, CBS
Alnicola escharoides, CBS
Boletus lutens, CBS
Boletus species, Peck Strain 168 Ohio State
Clavaria cristata, CBS
Clavaria ligula, CBS
Clitocybe tabescens, CBS
Collypia velutipes, CBS
Coniophora cerebella, CBS
Coprinus narcoticus, CBS
Corticium microsclerotia, NRRL 2705
Corticium sasakii, NRRL 2727
Deconia coprophilia, CBS
Entaloma sericeum, CBS
Fomes applanata, CBS
Hygrophorus protensis, CBS
Lentinellus ursinus, ATCC 11779
Panacolus campestris, New York Botanical, Garden St. L. 371
Paxillus acheruntius, CBS
Peniophora macrospora, CBS
Pholiota adiposa, CBS
Pholiota aegerita, CBS
Plicatura faginea, CBS
Polyporus hirsutus, CBS
Poria ambigua, ATCC 9408
Psalliota campestris, CBS
Schizophyllum commune, CBS
Sparassis crispa, CBS
Stereum fasciatum, CBS
Stereum rameale, CBS
Tricholoma inamoenum, CBS
Trametes hispida, CBS
Lycoperdon gemmatum, CBS
Sphaerobolus stellatus, NRRL 2922
Crucibulum vulgare, CBS
Cyathus olla, CBS

Cyathus poeppigi, CBS
Cyathus striatus, CBS
Ascochyta linicola, NRRL 2923
Coniothyrium tuckellii, ATCC 11349
Dendrophroma faginca, CBS
Diplodia natalensis, ATCC 9055
Diplodina coloradensis, CBS
Dendrophroma pleurospora, CBS
Polylopeus purpurius, CBS
Sphaeropsis visci
Endomosporium maculatum, CBS
Pestalotia guepini, ATCC 11543
Septomyxa affinis, ATCC 3737
Steganosporium piriforme, CBS
Aspergillus nidulans, ATCC 11267
Tricothecium roseum, ATCC 8685
Curvularia lunata, ATCC 12017
Cylindrocarpon didymum, CBS
Cylindrocarpon album, CBS
Helminthosporium carbonum, ATCC 9627
Penicillium atrovenetum, CBS
Penicillium patulum, ATCC 9260
Sepedonium ampullosporum, NRRL 2877
Sporotrichum bombycinum, ATCC 7137
Sporotrichum epigaeum, ATCC 7145
Sporotrichum sulphurescens, ATCC 7159
Papulospora polyspora, CBS
Rhizoctonia solani, ATCC 10159

EXAMPLE 2.

Bioconversion of Cyclododecanol

A medium was prepared of 50 g. of dried malt extract and 5 g. of commercial peptone, diluted to 1 l., normal pH about 6.5. 1 ml. of lard oil was added to an antifoam preventive. 100 ml. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Cylindrocarpon radicicola*, A.T.C.C. 11011, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 to 48 hours, or when a moderate to heavy growth of mycelium was apparent by visual observation, of agitation, a solution of 20 mg. of cyclododecanol in 1 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium (the whole culture) was extracted 4 times with a volume of methylene chloride equal to about one-fourth the volume of the whole culture. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue. The residue thus obtained was assayed by paper strip chromatography by the process described in Example 1 and found to consist of mixtures or dioxygenated cyclododecanes in the several oxidation states, namely,

cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone, and
7-hydroxycyclododecanone.

The crude residue thus obtained was dissolved in 1 ml. of acetone and oxidized at room temperature by the addition of a visible excess of Jones chromic acid reagent. The excess oxidant was destroyed by the addition of a few drops of isopropyl alcohol and the mixture evaporated to dryness. Water (2 ml.) was added, and the products were extracted with 2 ml. of methylene chloride. The extract was evaporated to dryness and the residue subjected to paper chromatography and gas-liquid (vapor phase) chromatography in accordance with the processes described in Example 1. Cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione were found to be present.

EXAMPLE 3.

Bioconversion of Cyclododecanol

A medium was prepared of 50 g. of dried malt extract and 5 g. of commercial peptone, diluted to 1 l., normal pH about 6.5. 1 ml. of lard oil was added as an antifoam preventive. 100 ml. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Chaetomium globosum*, A.T.C.C. 6205, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 to 48 hours, or when a moderate to heavy growth of mycelium was apparent by visual observation, of agitation, a solution of 20 mg. of cyclododecanol in 1 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium (the whole culture) was extracted 4 times with a volume of methylene chloride equal to about one-fourth the volume of the whole culture. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue. The residue thus obtained was assayed by paper strip chromatography by the process described in Example 1 and found to consist of mixtures of dioxygenated cyclododecanes in the several oxidation states, namely,

cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone, and
7-hydroxycyclododecanone.

The crude residue thus obtained was dissolved in 1 ml. of acetone and oxidized at room temperature by the addition of a visible excess of Jones chromic acid reagent. The excess oxidant was destroyed by the addition of a few drops of isopropyl alcohol and the mixture evaporated to dryness. Water (2 ml.) was added, and the products were extracted with 2 ml. of methylene chloride. The extract was evaporated to dryness and the residue subjected to paper chromatography and gas-liquid (vapor phase) chromatography in accordance with the processes described in Example 1. Cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione were found to be present.

EXAMPLE 4.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor (60 percent solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 100 ml. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Lentinas tigrinus*, A.T.C.C. 9406, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 to 1. per minute at 300 r.p.m. After 24 to 48 hours, or when a moderate to heavy growth of mycelium was apparent by visual observation, of agitation, a solution of 20 mg. of cyclododecanol in 1 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium (the whole culture) was extracted 4 times with a volume of methylene chloride equal to about one-fourth the volume of the whole culture. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue. The residue thus obtained was assayed by paper strip chromatography by the process described in Example 1 and found to consist of mixtures of dioxygenated cyclododecanes in the several oxidation states, namely,

cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone, and
7-hydroxycyclododecanone.

The crude residue thus obtained was dissolved in 1 ml. of acetone and oxidized at room temperature by the addition of a visible excess of Jones chromic acid reagent. The excess oxidant was destroyed by the addition of a few drops of isopropyl alcohol

and the mixture evaporated to dryness. Water (2 ml.) was added, and the products were extracted with 2 ml. of methylene chloride. The extract was evaporated to dryness and the residue subjected to paper chromatography and gas-liquid (vapor phase) chromatography in accordance with the processes described in Example 1. Cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione were found to be present.

EXAMPLE 5.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor (60 percent solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 100 ml. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Pleurotus paseckerianus*, A.T.C.C. 9416, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 to 48 hours, or when a moderate to heavy growth of mycelium was apparent by visual observation, of agitation, a solution of 20 mg. of cyclododecanol in 1 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium (the whole culture) was extracted 4 times with a volume of methylene chloride equal to about one-fourth the volume of the whole culture. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue. The residue thus obtained was assayed by paper strip chromatography by the process described in Example 1 and found to consist of mixtures of dioxygenated cyclododecanes in the several oxidation states, namely,

cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone, and
7-hydroxycyclododecanone.

The crude residue thus obtained was dissolved in 1 ml. of acetone and oxidized at room temperature by the addition of a visible excess of Jones chromic acid reagent. The excess oxidant was destroyed by the addition of a few drops of isopropyl alcohol and the mixture evaporated to dryness. Water (2 ml.) was added, and the products were extracted wtih 2 ml. of methylene chloride. The extract was evaporated to dryness and the residue subjected to paper chromatography and gas-liquid (vapor phase) chromatography in accordance with the processes described in Example 1. Cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione were found to be present.

EXAMPLE 6.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 2.0 g. of cyclododecanol in 20 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the washwater was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing cyclododecane-1,5-dione, cyclododecane-1,6-dione, cyclododecane-1,7-dione, 5-hydroxycyclododecanone, 6-hydroxycyclododecanone, 7-hydroxycyclododecanone, cyclododecane-1,5-diol, cyclododecane-1,6-diol and cyclododecan-1,7-diol.

Chromatography of this residue thus obtained over a column of synthetic magnesium silicate (Florisil—Registered Trade Mark) (3.8 × 35 cm.) packed in Skellysolve B hexanes, taking 335 ml. eluate fractions (unless otherwise noted) gave the following result:

Fraction	Eluting Solvent	Residue Wt.
1	Skellysolve B hexanes (1 liter)	25 mg.
2	5% Acetone-Skellysolve B hexanes	2
3	5% Acetone-Skellysolve B hexanes	26
4	5% Acetone-Skellysolve B hexanes	118
5	10% Acetone-Skellysolve B hexanes	68
6	10% Acetone-Skellysolve B hexanes	70
7	10% Acetone-Skellysolve B hexanes	260
8	10% Acetone-Skellysolve B hexanes	348
9	10% Acetone-Skellysolve B hexanes	169
10	10% Acetone-Skellysolve B hexanes	60
11	25% Acetone-Skellysolve B hexanes	19
12	25% Acetone-Skellysolve B hexanes	54
13	25% Acetone-Skellysolve B hexanes	66
14	25% Acetone-Skellysolve B hexanes	41
15	25% Acetone-Skellysolve B hexanes	23
16	25% Acetone-Skellysolve B hexanes	18
17	Acetone	56
18	Acetone	87
19	Acetone	13

Fraction 8 was recrystallized to give 0.14 g. of a mixture of 6 - hydroxycyclododecanone and 7 - hydroxycyclododecanone, of melting point 66—67°. The infrared spectrum showed absorption bands corresponding to the presence of hydroxyl and carbonyl functions in this material. The analytical sample crystallized from Skellysolve B hexanes: m.p. 68—69°.

Anal. Calcd. for $C_{12}H_{22}O_2$: C, 72.68;H, 11.18.
Found: C, 73.00; H, 11.12.

Oxidation of the hydroxyketone mixture thus obtained in acetone with excess 2.67 *M* aqueous chromic acid (Jones reagent: prepared by dissolving 26.72 g. of chromium trioxide in 23.0 ml. of concentrated sulfuric acid and 100 ml. of water) gave a crystalline diketone mixture, sublimed at reduded pressure to give a mixture of 1,6- and 1,7-cyclododecanediones, m.p. 63—64°.

Fractions 4 and 5 from the above chromatogram contained the cyclododecanedione mixture, containing cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione, from which, by chromatography over a column of Florisil packed in Skellysolve B hexanes, the isomeric diones can be obtained separately in the manner described in the following examples.

In the same manner the other microorganisms named in Examples 1, 2, 3, 4 and 5, can be substituted in place of *Sporotrichum sulphurescens* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 7.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 100 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 25°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 20.0 g. of cyclododecanol in 200 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the washwater was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing the same bioconversion products listed in Example 6 above.

This crude residue was then dissolved in 100 ml. of acetone and oxidized with excess chromic acid (Jones reagent) (about 30 ml.) at 35—40°C. The reaction mixture was stirred for about 10 minutes and then the excess chromic acid was destroyed by adding 10 ml. of isopropanol. The reaction mixture was then diluted with 250 ml. of water and extracted with three 150 ml. portions of methylene chloride and then with four 100 ml. portions of methylene chloride. The combined extracts were washed with 150 ml. of water, dried over anhydrous sodium sulfate and the solvent removed by distillation under reduced pressure to give about 20 g. of a semi-crystalline residue containing cyclododecane-1,5-dione, cyclododecane-1,6-dione and cyclododecane-1,7-dione.

The residue thus obtained was dissolved in benzene and chromatographed over a 7.5 × 35 cm. column of synthetic magnesium silicate (Florisil—Registered Trade Mark). Elution was with 2 liter portions of solvent as follows:

Fraction	Solvent	Residue Wt.
1	Skellysolve B hexanes	26 mg.
2	Skellysolve B hexanes	0
3	2% Acetone-Skellysolve B hexanes	1
4	2% Acetone-Skellysolve B hexanes	2853
5	5% Acetone-Skellysolve B hexanes	5149
6	5% Acetone-Skellysolve B hexanes	1908
7	5% Acetone-Skellysolve B hexanes	2008
8	10% Acetone-Skellysolve B hexanes	2306
9	10% Acetone-Skellysolve B hexanes	800
10	Acetone	3443

Paper chromatographic analysis of fractions 4, 5, 6, 7, 8, and 10 showed the following approximate compositions, expressed as percentages of total sample applied to the chromatograms.

Fraction	1,5-Dione	1,6-Dione	1,7-Dione
4	22.5	45	0
5	10	45	0
6	0	45	16
7	0	16	45
8	0	4	45
10	0	0	0

Fraction 4 was rechromatographed (see below). Fractions 7 to 9 inclusive were combined and recrystallized from acetone-Skellysolve B hexanes to give 2.32 g. of cyclododecane-1,7-dione, m.p. 132—135°. Further recrystallization of this substance from the same solvent system afforded an analytical sample of cyclododecane-1,7-dione, m.p. 134—136°.

Anal. Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27.
Found: C, 73.66; H, 9.99.

Fraction 4 (see above) was rechromatographed on a 3.8 × 35 cm. column of Florisil (Registered Trade Mark), eluting with 335 ml. fractions, except as noted:

Fraction	Solvent	Residue Wt.
1	Skellysolve B hexanes (1 l.)	0 mg.
2	1% Acetone-Skellysolve B hexanes	11
3	1% Acetone-Skellysolve B hexanes	14
4	1% Acetone-Skellysolve B hexanes	232
5	1% Acetone-Skellysolve B hexanes	659
6	1% Acetone-Skellysolve B hexanes	530
7	1% Acetone-Skellysolve B hexanes	355
8	2% Acetone-Skellysolve B hexanes	266
9	2% Acetone-Skellysolve B hexanes	262
10	2% Acetone-Skellysolve B hexanes	139
11	2% Acetone-Skellysolve B hexanes	46
12	2% Acetone-Skellysolve B hexanes	23
13	2% Acetone-Skellysolve B hexanes	0

Fractions 4, 5, and 6 of this chromatogram and fraction 5 of the earlier chromatogram were rechromatographed over alumina to obtain cyclododecane-1,5-dione.

Illustrative of this process is the chromatogram of fraction 5 on 50 g. of Merck Reagent (Registered Trade Mark) alumina. Elution was effected with 13 fractions of 1% acetone-Skellysolve B hexanes totalling 500 ml. Fractions 4—12 were crystalline solids having melting points as follows: fraction 4, 59—62°; fraction 5, 59—63°; fraction 6, 57—58°; fraction 7, 56—62°; fraction 8, 67—75°; and fraction 11, 91—92°. Fraction 4 appeared to be mainly one component, the 1,5-dione, with later fractions containing increasing amounts of the 1,6-dione. Fractions 4—7, totalling 0.224 g., were combined for rechromatography on 25 g. of Merck (Registered Trade Mark) alumina. Elution was effected with 0.5% acetone-Skellysolve B hexanes in fractions 1—15, totalling 100 ml. of eluting solvent, followed by elution with 1% acetone-Skellysolve B hexanes in fractions 11—21, totalling 250 ml. of eluting solvent. From this chromatogram fractions 1—6, all of which melted below 60° and which totalled 110 mg., were combined and crystallized from hexane to give 50 mg. of cyclododecane-1,5-dione, melting point 64—65°.

Anal. Calcd. for $C_{12}H_{20}O_2$: C, 73.42; H, 10.27.
Found: C, 73.91; H, 10.19.

In the same manner the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Sporotrichum sulphuescens* to give the same products, differing only in the relative amounts of the position isomers produced.

Example 8.

Bioconversion of cyclododecanol

A medium was prepared of 20 g. of cornsteep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventative. 125 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 25.0 g. of cyclododecanol in 250 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the washwater was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing the same bioconversion products as listed in Example 6, above.

The residue thus obtained was dissolved in methylene chloride and chromatographed on a 7.5 × 35 cm. column of synthetic magnesium silicate (Florisil—Registered Trade Mark) packed in Skellysolve B hexanes. Elution was with 2 liter portions of solvent as follows:

Fraction	Eluting Solvent	Residue Wt.
1	Skellysolve B hexanes	275 mg.
2	Skellysolve B hexanes	0
3	2% Acetone-Skellysolve B hexanes	0
4	2% Acetone-Skellysolve B hexanes	197
5	2% Acetone-Skellysolve B hexanes	3914
6	2% Acetone-Skellysolve B hexanes	1503
7	5% Acetone-Skellysolve B hexanes	334
8	5% Acetone-Skellysolve B hexanes	2285
9	5% Acetone-Skellysolve B hexanes	2384
10	5% Acetone-Skellysolve B hexanes	3262
11	10% Acetone-Skellysolve B hexanes	2772
12	10% Acetone-Skellysolve B hexanes	3274
13	10% Acetone-Skellysolve B hexanes	442
14	10% Acetone-Skellysolve B hexanes	161

Fractions 5, 6 and 7 of this chromatogram were combined and crystallized from acetone-Skellysolve B hexanes to give 2.81 g. of cyclododecane-1,6-dione, m.p. 91—95°. For analysis a sample was recrystallized twice from acetone-Skellysolve B hexanes to give cyclododecane-1,6-dione, m.p. 94.5—95.5°C.

Anal. Calcd. for $C_{12}H_{20}O_2$: C, 73.43; H, 10.27.
Found: C, 73.64; H, 9.99.

In the same manner the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Sporotrichum sulphurescens* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 9.

Bioconversion of cyclododecanol

A medium was prepared of 20 g. of corn steep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to apH of 5.0. 0.2 ml. of Dow-Corning (Registered Trade Mark) C—120 oil was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 96-hour vegetative growth of *Ascochyta linicola*, NRRL 2923 and incubated for 48 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 48 hours of agitation, a solution of 2.5 g. of cyclododecanol in 25 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 48-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the washwater was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing 5-hydroxycyclododecanone, 6-hydroxycyclododecanone, 7-hydroxycyclododecanone, cyclododecane-1,5-diol, cyclododecane-1,6-diol and cyclododecane-1,7-diol.

The extract residue was chromatographed over Florisil—Registered Trade Mark. Materials eluted by 2—5% acetone-Skellysolve B hexanes and by acetone were oxidized separately with chromic acid and analyzed paper chromatographically. No appreciable amounts of cyclododecanediones were found. Material eluted by 10% acetone-Skellysolve B hexanes was recrystallized from acetone-Skellysolve B hexanes to give 0.28 g. of cyclododecanolones, m.p. 87—90°. Oxidation of this material, as well as of the mother liquor residue, afforded mixtures of 1,6- and 1,7-cyclododecanedione, as determined by paper chromatography. Vapor phase chromatography by the method described in Example 1 also showed that cyclododecanone was present in the oxidized samples, indicating that cyclododecanol was present in the bioconversion product.

Material eluted from the Florisil — Registered Trade Mark — column with 25% acetone-Skellysolve B hexanes was recrystallized from acetone-Skellysolve B hexanes to give 0.39 g. of 1,6- and 1,7-cyclododecanediols, m.p. 122—135°, with no carbonyl absorption in the infrared spectrum. Oxidation of this material, as well as of the mother liquor residue, gave the same results as outlined above for the cyclododecanolones determined by paper strip chromatography according to the method described in Example 1, above.

In the same manner, the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Ascochyta linicola.*

EXAMPLE 10.

Bioconversion of Cyclododecanol

A medium was prepared of 20 g. of corn steep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 4.95. 0.2 ml. of Dow-Corning (Registered Trade Mark) C—120 oil was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Absidia glauca* A.T.C.C. 7852a and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.3 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 2.5 g. of cyclododecanol in 25 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 48-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the wash water was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing the same bioconversion products as listed in Example 6, above.

The extract residue was chromatographed over Florisil. Material eluted with 10—25% acetone-Skellysolve B hexanes was recrystallized from acetone-Skellysolve B hexanes to give 0.33 g. of 6- and 7-hydroxycyclododecanones, m.p. 67—69°. An oxidized sample, analyzed by paper strip chromatography by the method described in Example 1, showed that 1,6-cyclododecanedione and 1,7-cyclododecanedione were present in about equal amounts.

All other eluates (5%, 10% and 25—100% acetone-Skellysolve B hexanes) from the Florisil (Registered Trade Mark) column were pooled and oxidized to a mixture of 1,6- and 1,7-cyclododecanediones contaminated with cyclododecanone (analyzed by paper strip chromatograph as above).

In the same manner, the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Absidia glauca* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 11.

Bioconversion of cyclotridecanol

A medium was prepared of 20 g. of corn steep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 5.0. 1 ml. of lard oil was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 2.0 of cyclotridecanol in 20 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the wash water was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was

removed by distillation to give a crude residue containing cyclotridecane-1,5-dione, cyclotridecane-1,6-dione, cyclotridecane-1,7-dione, 5-hydroxycyclotridecanone, 6-hydroxycyclotridecanone, 7-hydroxycyclotridecanone, cyclotridecane-1,5-diol, cyclotridecane-1,6-diol and cyclotridecane-1,7-diol.

The extract residue thus obtained was dissolved in 15 ml. of acetone and oxidized with excess 2.57 *M* chromic acid (Jones reagent) keeping the temperature between 35 and 40°C. The reaction was stirred for 10 minutes and then 2 ml. of isopropanol was added. The mixture was then extracted 3 times with 20 ml. portions of methylene chloride. The extracts were combined, washed with 20 ml. of water, dried over anhydrous sodium sulphate, and distilled under vacuum to remove the solvent to give a semi-crystalline residue containing cyclotridecane - 1,5- dione, cyclotridecane-1,6-dione and cyclotridecane-1,7-dione.

The extract residue thus obtained was dissolved in benzene and chromatographed over 200 g. of synthetic magnesium silicate (Florisil — Registered Trade Mark). The column was eluted with thirty-four 250 ml. fractions of Skellysolve B hexanes containing increasing proportions of acetone, ranging from 0 to 6%. The product fractions were crystallized from ether - hexane to give 95 mg. of product, m.p. 88—90°. The major component of this material was cyclotridecane - 1,7 - dione with a smaller amount of cyclotridecane - 1,6 - dione and cyclotridecane - 1,5 - dione.

Anal. Calcd. for $C_{13}H_{22}O_2$: C, 74.24; H, 10.54.
Found: C, 74.09; H, 10.24.

In the same manner, the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Sporotrichum sulphurescens* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 12.

Bioconversion of Cyclotetradecanol

A medium was prepared of 20 g. of corn steep liquor (60% solids) and 10 g. of commercial dextrose, diluted to 1 l. and adjusted to a pH of 5.0. 0.2 ml. of Dow-Corning (Registered Trade Mark) C—120 was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 2.0 g of cyclotetradecanol in 20 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the wash water was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing cyclotetradecane - 1,5 - dione, cyclotetradecane - 1,6-dione, cyclotetradecane - 1,7 - dione, cyclotetradecane - 1,5 - diol, cyclotetradecane-1,6 - diol, cyclotetradecane - 1,7 - diol, 5 - hydroxycyclotetradecanone, 6 - hydroxycyclotetradecanone, and 7-hydroxycyclotetradecanone.

The extract residue from the bioconversion was oxidized with excess chromic acid, extracted and freed of solvent in the same manner as described in Example 5 above, and the oxidized product containing cyclotetradecane - 1,5 - dione, cyclotetradecane-1,6 - dione and cyclotetradecane - 1,7 - dione was then chromatographed in benzene over 200 g. of Florisil (Registered Trade Mark), eluting with thirty-four 250 ml. fractions of Skellysolve B hexanes containing increasing proportions of acetone, ranging from 0—6%. Fractions 4—6 contained 0.451 g. of cyclotetradecanone resulting from unconverted starting material. Fractions 14—20 contained 0.22 g. of crystalline product that was recrystallized from acetone - hexane to give 120 mg. of cyclotetradecane-1,6 - dione containing a small amount of cyclotetradecane - 1,7 - dione and of cyclotetradecane - 1,5 - dione. The melting point of the sample was 92°.

Anal. Calcd. for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78.
Found: C, 75.20; H, 10.94.

In the same manner, the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Sporotrichum sulphurescens* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 13.

Bioconversion of Cycloundecanol

A medium was prepared of 20 g. of corn steep liquor and 10 g. of commercial dextrose diluted to 1 l. and adjusted to a pH of 4.85. 1 ml. of lard oil was added as an antifoam preventive. 10 l. of this sterilized medium was inoculated with a 72-hour vegetative growth of *Sporotrichum sulphurescens* A.T.C.C. 7159, and incubated for 24 hours at a temperature of about 28°C. using a rate of aeration of 0.5 l. per minute at 300 r.p.m. After 24 hours of agitation, a solution of 2.0 g. of cycloundecanol in 20 ml. of N,N - dimethylformamide was added to the inoculated medium. After an additional 72-hour period of incubation, the beer and mycelium were separated by filtration. The mycelium was washed with water and the wash water was added to the beer filtrate. The thus-obtained beer filtrate was extracted 4 times with a volume of methylene chloride equal to one-fourth the volume of the filtrate. The combined extracts were washed with one-fourth volume of distilled water and the solvent was removed by distillation to give a crude residue containing cycloundecane-1,5-dione, cycloundecane-1,6-dione, 5-hydroxycycloundecanone, 6-hydroxycycloundecanone, cycloundecane-1,5-diol, and cycloundecane-1,6-diol.

The crude residue thus obtained is then dissolved in acetone and oxidized with excess chromic acid (Jones reagent). The reaction mixture is stirred for about 10 minutes and then the excess chromic acid was destroyed by adding 10 ml. of isopropanol. The reaction mixture is then diluted with about 250 ml. of water and extracted with three 150 ml. portions of methylene chloride and then with four 100 ml. portions of methylene chloride. The combined extracts are washed with 150 ml. of water, dried over anhydrous sodium sulphate and the solvent removed by distillation under reduced pressure to give a semi-crystalline residue containing cycloundecane-1,5-dione and cycloundecane-1,6-dione.

This residue is then dissolved in benzene and chromatographed over a 7.5 × 35 cm. column of synthetic magnesium silicate (Florisil—Registered Trade Mark) and eluted with increasing proportions (2 to 10%) of acetone in Skellysolve B hexanes.

The fractions containing cycloundecane-1,5-dione (determined by infrared analysis) are combined and distilled to remove the solvent to give a residue of cycloundecane-1,5-dione which can be purified by crystallization from acetone-hexanes to give cycloundecane-1,5-dione, a light colored crystalline solid.

The fractions containing cycloundecane-1,6-dione (determined by infrared analysis) are likewise combined and distilled to remove the solvent to give a residue of cycloundecane-1,6-dione, which can be purified by crystallization from acetone-hexanes to give cycloundecane-1,6-dione, a light colored crystalline solid.

In the same manner, the other microorganisms named in Examples 1, 2, 3, 4 and 5 can be substituted in place of *Sporotrichum sulphurescens* to give the same products, differing only in the relative amounts of the position isomers produced.

EXAMPLE 14.

6-hydroxycyclododecanone and cyclododecane-1,6-diol

A stirred solution of 0.98 g. of cyclododecane-1,6-dione in 20 ml. of tetrahydrofuran under nitrogen was chilled in an ice-methanol bath and a solution of 1.4 g. of lithium tritertiarybutoxy aluminum hydride in 20 ml. of tetrahydrofuran was added rapidly from a dropping funnel. The reaction mixture was stirred in the cold bath for 15 minutes, at room temperature for 15 minutes and then poured onto ice and neutralized to pH 6—7 with hydrochloric acid. The aqueous mixture was extracted 3 times with 20 ml. portions of ether, the ether extracts were combined, washed with water, dried over anhydrous sodium sulphate and the solvent removed by evaporation to give a semi-crystalline residue. The residue thus obtained was dissolved in 35 ml. of benzene and chromatographed over a column containing 50 g. of synthetic magnesium silicate (Florisil — Registered Trade Mark) and eluted with 500 ml. portions of solvent as follows:

Fraction	*Eluting Solvent*
1—6	2% acetone-Skellysolve B hexanes
7—11	4% acetone-Skellysolve B hexanes
12—16	6% acetone-Skellysolve B hexanes
17—21	8% acetone-Skellysolve B hexanes
22—23	20% acetone-Skellysolve B hexanes

The solvent was evaporated from each of the fractions. Fractions 2—7 gave 0.378 g. of starting material, fractions 10—16 gave 0.476 g. of 6 - hydroxycyclodedecanone and fractions 21—22 gave 0.030 g. of cyclododecane-1,6-diol, determined by infrared analysis.

Fractions 10—16 were combined and crystallized from ether pentane to give 0.466 g. of 6-hydroxycyclododecanone, m.p. 69—69°C. Infrared analysis confirmed the structure.

Anal. Calcd. for $C_{12}H_{22}O_2$: C, 72.68; H, 11.19.
Found: C, 72.81; H, 11.51.

EXAMPLE 15.

7-hydroxycyclododecanone and cyclododecane-1,7-diol

A solution of 0.98 g. of cyclododecane-1,7-dione in 40 ml. of ether was stirred and treated dropwise over a period of 10 minutes with a solution of 6.2 ml. of 1.6*N* ethereal primary isobutyl magnesium bromide diluted with 20 ml. of ether. After an additional 10 minute reaction period, 10 ml. of water was continuously added and the mixture was acidified with 2 *N* sulfuric acid. The mixture was then extracted with ether. The extracts were combined, washed with water, dried over anhydrous sodium sulphate and the solvent removed by evaporation to give 0.987 g. of a semi-crystalline residue. The residue, thus obtained, was dissolved in benzene and chromatographed over a column of 50 g. of synthetic magnesium silicate (Florisil — Registered Trade Mark) and eluted with 500 ml. portions of solvent as follows:

Fractions	*Eluting solvent*
1—5	2% acetone-Skellysolve B hexanes
6—10	4% acetone-Skellysolve B hexanes
11—15	6% acetone-Skellysolve B hexanes
16—20	10% acetone-Skellysolve B hexanes

Fractions 11—15 contained 0.230 g. of 7-hydroxycyclododecanone which was crystallized from acetone-hexanes and then from ether-pentane to give 7-hydroxy-cyclododecanone, m.p. 94°C.

Anal. Calcd. for $C_{12}H_{22}O_2$: C, 72.68; H, 11.19.
Found: C, 72.68; H, 11.38.

Fractions 16—20 contained 0.091 g. of cyclododecane-1,7-diol, m.p. 168—175.

Anal. Calcd. for $C_{12}H_{24}O_2$: C, 71.95; H, 12.08.
Found: C, 72.01; H, 11.85.

EXAMPLE 16.

7-hydroxycyclotetradecanone

A stirred solution of 1.12 g. of cyclotetradecane-1,7-dione in 40 ml. of ether was cooled in an ice bath and a solution of 6.2 ml. of 1.6 *N* ethereal primary isobutyl magnesium bromide diluted with 15 ml. of ether was added dropwise. The precipitate thus obtained was collected on a filter, washed with ether and dried. The dry product was then mixed with 50 ml. of dilute hydrochloric acid and extracted 3 times with 25 ml. portions of ether. The ether extracts were combined, washed with water, dried over anhydrous sodium sulphate and the solvent removed to give 1.10 g. of an oil which was diluted with 35 ml. of benzene and chromatographed over a column containing 25 g. of synthetic magnesium silicate (Florisil) and eluted with solvent as follows:

Fractions	*Eluting Solvent*
1—5	1% acetone-Skellysolve B hexanes
6—10	2% acetone-Skellysolve B hexanes
11—15	3% acetone-Skellysolve B hexanes
16—20	4% acetone-Skellysolve B hexanes
21—24	6% acetone-Skellysolve B hexanes
25—27	8% acetone-Skellysolve B hexanes
28—29	10% acetone-Skellysolve B hexanes

The fractions were analyzed by infrared, the solvent was removed from fractions 11—19 to give 0.256 g. of 7-hydroxycyclotetradecanone, which was crystallized from acetone-hexane to give 7-hydroxycyclotetradecanone, m.p. 83—84°C.

Anal. Calcd. for $C_{24}H_{26}O_2$: C, 74.28; H, 11.58.
Found: C, 74.48; H, 11.53.

EXAMPLE 17.

Cyclotetradecane-1,7-diol

To a mixture of 100 ml. of anhydrous ether and 2.0 g. of lithium aluminum hydride was added a solution of 2.05 g. of cyclotetradecane-1,7-dione in 40 ml. of ether. The mixture was stirred at about 28°C. for about 30 minutes and then water was added dropwise until decomposition of the excess hydride was complete. The mixture was then diluted with 200 ml. of ether, filtered and the filtrate dried over anhydrous sodium sulphate. The solvent was removed by distillation to give a solid residue of cyclotetradecane-1,7-diol, m.p. 111—112°C.

Anal. Calcd. for $C_{24}H_{28}O_2$: C, 73.63; H, 12.36.
Found: C, 73.61; H, 12.07.

In the same manner, substituting as starting material
cycloundecane-1,5-dione,
cycloundecane-1,6-dione,
cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclotridecane-1,5-dione,
cyclotridecane-1,6-dione,
cyclotridecane-1,7-dione,
cyclotetradecane-1,5-dione,
cyclotetradecane-1,6-dione, or
cyclotetradecane-1,7-dione,
Example 17 is productive of:
cycloundecane-1,5-diol,
cycloundecane-1,6-diol,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
cyclotridecane-1,5-diol,
cyclotridecane-1,6-diol,
cyclotridecane-1,7-diol,
cyclotetradecane-1,5-diol,
cyclotetradecane-1,6-diol, and
cyclotetradecane-1,7-diol, respectively.

EXAMPLE 18.

5-hydroxycyclododecanone and cyclododecane-1,5-diol

A stirred solution of 1.0 g. of cyclododecane-1,5-dione in ether is stirred and treated dropwise with a solution of 6.2 ml. of 1.6 *N* primary isobutyl magnesium bromide diluted with 20 ml. of ether. The reaction mixture is stirred for about 10 to 15 minutes or until the reaction is essentially complete, 10 ml. of water is then added cautiously and the mixture acidified with 2 *N* sulphuric acid. The mixture is then extracted with ether, the extracts combined, washed with water, dried over sodium sulphate and the solvent removed to give a residue of cyclododecane-1,5-diol. The residue thus obtained is dissolved in benzene and chromatographed over a column of synthetic magnesium silicate (Florisil — Registered Trade Mark). The column was eluted with increasing proportions (2 to 10%) of acetone in Skellysolve B hexanes.

The fractions containing 5-hydroxycyclododecanone, determined by infrared analysis, are combined and distilled to remove the solvent, crystallization of the residue thus obtained from acetone-pentane gives 5-hydroxycyclododecanone, a light colored crystalline solid.

The fractions containing cyclododecane-1,5-diol, determined by infrared analysis, are combined and distilled to remove the solvent, crystallization of the residue thus

obtained from acetone-hexane gives cyclododecane-1,5-diol, a light colored crystalline solid.

In the same manner subsitituting as starting material

cycloundecane-1,5-dione,
cycloundecane-1,6-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclotridecane-1,5-dione,
cyclotridecane-1,6-dione,
cyclotridecane-1,7-dione,
cyclotetradecane-1,5-dione,
cyclotetradecane-1,6-dione, or
cyclotetradecane-1,7-dione,

Example 18 is productive of

5-hydroxyundecanone,
6-hydroxycycloundecanone,
6-hydroxycyclododecanone,
7-hydroxycyclododecanone,
5-hydroxycyclotridecanone,
6-hydroxycyclotridecanone,
7-hydroxycyclotridecanone,
5-hydroxycyclotetradecanone,
6-hydroxycyclotetradecanone,
7-hydroxycyclotetradecanone, and

the corresponding cycloalkane-diols, respectively.

EXAMPLE 19.

Hydroxycyclododecanone Hemisuccinates and the Sodium Salts Thereof

A mixture of 2.0 g. of 6-hydroxycyclododecanone and 7-hydroxycyclododecanone, 2.06 g. of succinic anhydride and 10 ml. of pyridine was refluxed for 1 hour, poured into water, acidified to pH 1 with 3 *N* sulphuric acid, and extracted with methylene chloride. The extracts were washed with water and evaporated to dryness. The residue was partitioned between methylene chloride and aqueous 5% sodium bicarbonate, and the aqueous phase, after washing with fresh methylene chloride, was acidified to pH 1 with *N* hydrochloric acid and extracted with methylene chloride. The extract, after drying and evaporation of the solvent, gave 0.97 g. of an oil that crystallized. Recrystallization from acetone Skellysolve B hexanes afforded 0.55 g. of a mixture of 6-hydroxycyclododecanone hemisuccinate and 7-hydroxycyclododecanone hemisuccinate, m.p. 83—94°.

Trituration of 0.5 of this material in acetone with N/10 sodium hydroxide to pH 7.5, followed by lyophilization, gave 0.48 g. of the sodium salt as a hygroscopic powder.

In the same manner substituting the purified 6-hydroxycyclododecanone or 7-hydroxycyclododecanone as starting material, Example 19 is productive of 6-hydroxycyclododecanone hemisuccinate, 7-hydroxycyclododecanone hemisuccinate and the corresponding sodium salts thereof, respectively.

In the same manner substituting as starting material the other compounds of Formula III i.e.,

5-hydroxycycloundecanone,
6-hydroxycycloundecanone,
5-hydroxycyclododecanone,
5-hydroxycyclotridecanone,
6-hydroxycyclotridecanone,
7-hydroxycyclotridecanone,
5-hydroxycyclotetradecanone,
6-hydroxycyclotetradecanone, or
7-hydroxycyclotetradecanone,

Example 19 is productive of the corresponding hemisuccinate and the sodium salt thereof, respectively.

EXAMPLE 20.

Cyclododecane-1,6-diol

A mixture of 2.0 g. of cyclododecane-1,6-diol, 4.12 g. of succinic anhydride and 20 ml. of pyridine is refluxed for 1 hour, poured into water, acidified to pH 1 with 3 *N* sulphuric acid, and extracted with methylene chloride. The extracts are washed with

water and evaporated to dryness. The residue is partitioned between methylene chloride and aqueous 5% sodium bicarbonate, and the aqueous phase, after washing with fresh methylene chloride, is acidified to pH 1 with *N* hydrochloric acid and extracted with methylene chloride. The extract, after drying and evaporation of the solvent, gives crude cyclododecane-1,6 diol dihemisuccinate. Recrystallization from acetone-Skellysolve B hexanes gives cyclododecane-1,6-diol dihemisuccinate, a light colored crystalline solid.

Trituration of 0.5 g. of this material in acetone with N/10 sodium hydroxide to pH 7.5, followed by lyophilization, gives the sodium salt as a hygroscopic powder.

In the same manner, substituting as starting material
cycloundecane-1,5-diol,
cycloundecane-1,6-diol,
cyclododecane-1,5-diol,
cyclododecane-1,7-diol,
cyclotridecane-1,5-diol,
cyclotridecane-1,6-diol,
cyclotridecane-1,7-diol,
cyclotetradecane-1,5-diol,
cyclotetradecane-1,6-diol, or
cyclotetradecane-1,7-diol
Example 20 is productive of the corresponding dihemisuccinate and the sodium salt thereof, respectively.

EXAMPLE 21.

6-hydroxycyclododecanone

A mixture of 2.0 g. of 6-hydroxycyclododecanone and 10 g. of acetic anhydride was heated on a steam bath for 1 hour. The mixture was then poured into water, the excess anhydride allowed to decompose. The precipitated product thus obtained was recovered by extraction with methylene chloride. The methylene chloride extract was washed with aqueous molar sodium hydroxide solution to remove the acids, then with water and evaporated to dryness to give 6 - hydroxycyclododecanone acetate, a light colored crystalline solid.

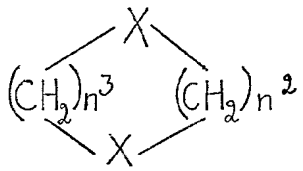
In the same manner substituting as starting material the compounds of Formula III, e.g., 5-hydroxycyclododecanone, 7-hydroxycyclododecanone, or the other compounds of Formula III listed in the last paragraphs of Example 19, Example 21 is productive of the corresponding acetate, i.e.,
5-hydroxycyclododecanone acetate,
7-hydroxycyclododecanone acetate,
5-hydroxycycloundecanone acetate,
6-hydroxycycloundecanone acetate,
5-hydroxycyclotridecanone acetate,
6-hydroxycyclotridecanone acetate,
7-hydroxycyclotridecanone acetate,
5-hydroxycyclotetradecanone acetate,
6-hydroxycyclotetradecanone acetate and
7-hydroxycyclotetradecanone acetate, respectively.

In the same manner substituting as starting material cyclododecane-1,6-diol or the other compounds of Formula II, listed in the last paragraph of Example 20, Example 21 is productive of
cycloundecane-1,5-diol diacetate,
cycloundecane-1,6-diol diacetate,
cyclododecane-1,5-diol diacetate,
cyclododecane-1,7-diol diacetate,
cyclotridecane-1,5-diol diacetate,
cyclotridecane-1,6-diol diacetate,
cyclotridecane-1,7-diol diacetate,
cyclotetradecane-1,5-diol diacetate,
cyclotetradecane-1,6-diol diacetate and
cyclotetradecane-1,7-diol diacetate, respectively.

In the same manner substituting in place of acetic anhydride, the acid anhydride of another hydrocarbon carboxylic acid containing from 1 to 12 carbon atoms, inclusive, for example, the acids previously listed, Example 21 is productive of the corresponding monoacylates and diacylates of the selected starting material, the products, thus obtained, corresponding otherwise to the mono- and diacetates named above.

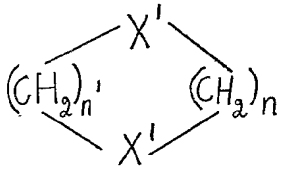
WHAT WE CLAIM IS:—

1. A dioxygenated cyclododecane of the formula:



wherein n^2 is a whole number from 5 to 7, inclusive and n^3 is a whole number from 3 to 5, inclusive, in which the sum of n^2+n^3+2 is 12, and wherein X in each case is $>C=O$ or $>C<^{OH}_{H}$, and may be the same or different.

2. Cyclododecane-1,5-dione.
3. Cyclododecane-1,6-dione.
4. Cyclododecane-1,7-dione.
5. Cyclododecane-1,5-diol.
6. Cyclododecane-1,6-diol.
7. Cyclododecane-1,7-diol.
8. 5-hydroxycyclododecanone.
9. 6-hydroxycyclododecanone.
10. 7-hydroxycyclododecanone.
11. A compound selected from the group consisting of cyclotridecane-1,5-diol, cyclotridecane-1,6-diol, cyclotridecane-1,7-diol, cyclotetradecane-1,5-diol, cyclotetradecane-1,6-diol, and cyclotetradecane-1,7-diol.
12. Cyclotetradecane-1,7-diol.
13. A compound selected from the group consisting of 5-hydroxycyclotridecanone, 6-hydroxycyclotridecanone, 7-hydroxycyclotridecanone, 5-hydroxycyclotetradecanone, 6-hydroxycyclotetradecanone, and 7-hydroxycyclotetradecanone.
14. 7-hydroxycyclotetradecanone.
15. A process for the introduction of oxygen into a cycloalkanol, having from 11 to 14 carbon atoms, inclusive, in the ring structure, which comprises: subjecting said cycloalkanol to the oxygenating activity of a species of microorganism of the subphylum Eumycetes to obtain a compound of the formula:



where X^1 in each case is $>C=O$ or $>C<^{OH}_{H}$ and may be the same or different, n is a whole number from 5 to 9, inclusive, n^1 is a whole number from 3 to 5 inclusive, and in which $n+n^1+2$ is not less than 11 and not more than 14.

16. A process as claimed in claim 15 wherein the fermentation is effected in an aqueous nutrient medium under submerged conditions and is continued until a substantial amount of oxygenated cycloalkanols is produced and the latter are then recovered from the fermentation medium.

17. The process of claim 15 or 16 wherein the microorganism is *Sporotrichum sulphurescens.*

18. The process of claim 15 or 16 wherein the microorganism is *Ascochyta linicola.*

19. The process of claim 15 or 16 wherein the microorganism is *Absidia glauca.*

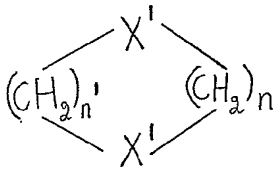
20. The process of claim 15 to 19 wherein the starting material is cycloundecanol.

21. The process of claim 15 to 19 wherein the starting material is cyclododecanol.

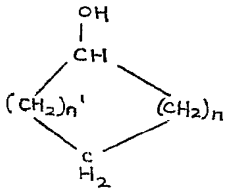
22. The process of claim 15 to 19 wherein the starting material is cyclotridecanol.

23. The process of claim 15 to 19 wherein the starting material is cyclotetradecanol.

24. A process for the production of a compound of the formula:



wherein X^1 in each case is $>C=O$ or $>C<_{H}^{OH}$ and may be the same or different, and wherein n is a whole number from 5 to 9, inclusive, n^1 is a whole number from 3 to 5, inclusive, and in which the sum of $n+n^1+2$ is not less than 11 and not more than 14, which comprises subjecting a compound of the formula:



wherein n and n^1 are defined as above, to the oxygenating activity of a species of the microorganisms of the subphylum Eumycetes in an aqueous nutrient medium under submerged fermentation conditions, continuing the fermentation until a substantial amount of the corresponding dioxygenated cycloalkanes is produced and recovering the dioxygenated cycloalkanone from the fermentation medium.

25. The process of claim 24 for the production of a mixture comprising cycloundecane-1,5-dione,
cycloundecane-1,6-dione,
cycloundecane-1,5-diol,
cycloundecane-1,6-diol,
5-hydroxycycloundecanone, and
6-hydroxycycloundecanone,
wherein the starting material is cycloundecanol.

26. The process of claim 24 for the production of a mixture comprising
cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone and
7-hydroxycyclododecanone
wherein the starting material is cyclododecanol.

27. The process of claim 24 for the production of a mixture comprising:
cyclotridecane-1,5-dione,
cyclotridecane-1,6-dione,
cyclotridecane-1,7-dione,
cyclotridecane-1,5-diol,
cyclotridecane-1,6-diol,
cyclotridecane-1,7-diol,

5-hydroxycyclotridecanone,
6-hydroxycyclotridecanone and
7-hydroxycyclotridecanone
wherein the starting material is cyclotridecanol.

28. The process of claim 24 for the production of a mixture comprising cyclotetradecane-1,5-dione,
cyclotetradecane-1,6-dione,
cyclotetradecane-1,7-dione,
cyclotetradecane-1,5-diol,
cyclotetradecane-1,6-diol,
cyclotetradecane-1,7-diol,
5-hydroxycyclotetradecanone,
6-hydroxycyclotetradecanone and
7-hydroxycyclotetradecanone
wherein the starting material is cyclotetradecanol.

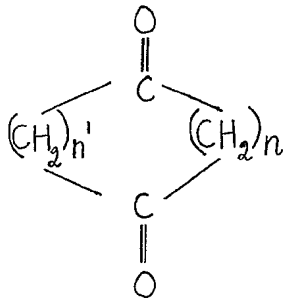
29. The process which comprises: aerobically contacting cyclododecanol with the oxygenating activity of the fungus *Sporotrichum sulphuresens* in an aqueous nutrient medium under submerged fermentation conditions and continuing the fermentation until a substantial amount of a mixture comprising:
cyclododecane-1,5-dione,
cyclododecane-1,6-dione,
cyclododecane-1,7-dione,
cyclododecane-1,5-diol,
cyclododecane-1,6-diol,
cyclododecane-1,7-diol,
5-hydroxycyclododecanone,
6-hydroxycyclododecanone and
7-hydroxycyclododecanone
is produced.

30. The process of claim 29, wherein the microorganism is *Absidia glauca.*

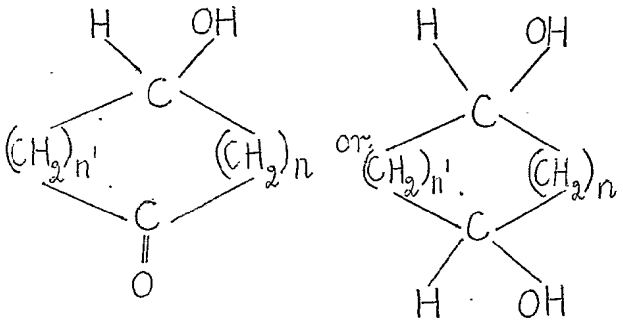
31. The process which comprises: aerobically contacting cyclotridecanol with the oxygenating activity of the fungus *Sporotrichum sulphuresens* in an aqueous nutrient medium under submerged fermentation conditions and continuing the fermentation until a substantial amount of a mixture comprising:
cyclotridecane-1,5-dione,
cyclotridecane-1,6-dione,
cyclotridecane-1,7-dione,
cyclotridecane-1,5-diol,
cyclotridecane-1,6-diol,
cyclotridecane-1,7-diol,
5-hydroxycyclotridecanone,
6-hydroxycyclotridecanone and
7-hydroxycyclotridecanone
is produced.

32. The process which comprises: aerobically contacting cyclotetradecanol with the oxygenating activity of the fungus *Sporotrichum sulphuresens* in an aqueous nutrient medium under submerged fermentation conditions and continuing the fermentation until a substantial amount of a mixture comprising:
cyclotetradecane-1,5-dione,
cyclotetradecane-1,6-dione,
cyclotetradecane-1,7-dione,
cyclotetradecane-1,5-diol,
cyclotetradecane-1,6-diol,
cyclotetradecane-1,7-diol,
5-hydroxycyclotetradecanone,
6-hydroxycyclotetradecanone,
7-hydroxycyclotetradecanone,
is produced.

33. The process for the production of a compound of the formula:

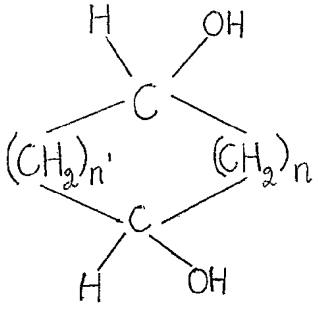


wherein n is a whole number from 5 to 9, inclusive, n′ is a whole number from 3 to 5, inclusive, and in which the sum of $n+n'+2$ is not less than 11 and not more than 14, which comprises reacting a compound of the formula:

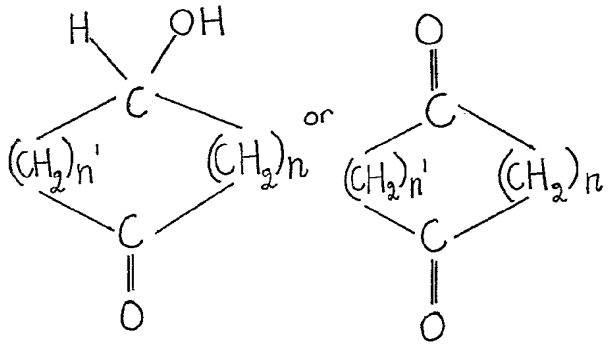


wherein n and n′ are defined as above, with an oxidizing agent to produce the corresponding cycloalkanedione.

34. The process for the production of a compound of the formula:

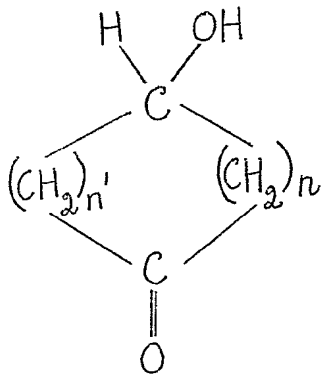


wherein n is a whole number from 5 to 9 inclusive, n′ is a whole number from 3 to 5, inclusive, and in which $n+n'+2$ is not less than 11 and not more than 14, which comprises reacting a compound of the formula:

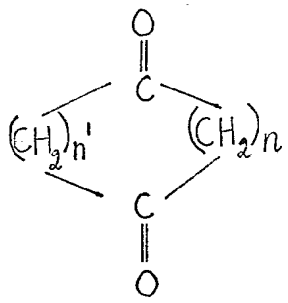


wherein n and n^1 are defined as above, with a carbonyl reducing agent to produce the corresponding cycloalkanediol.

35. The process for the production of a compound of the formula:

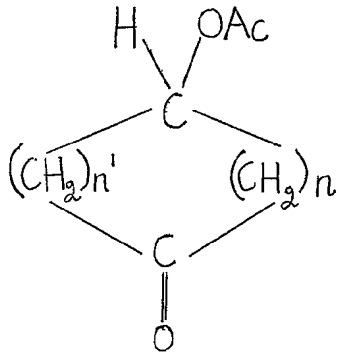


wherein hydrogen or n is a whole number from 5 to 9, inclusive, and n^1 is a whole number from 3 to 5, inclusive, and in which $n+n^1+2$ is not less than 11 and not more than 14, which comprises partially reducing a compound of the formula:



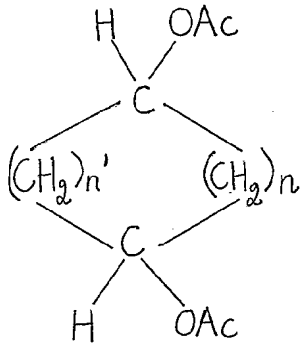
with a carbonyl reducing agent to produce the corresponding hydroxycycloalkanone.

36. A process for the production of a compound of the formula



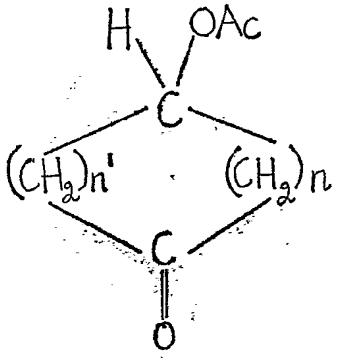
wherein Ac is the acyl radical of a hydrocarbon carboxylic acid containing from 1 to 12 carbon atoms inclusive, n is a whole number from 5 to 9 inclusive, n^1 is a whole number from 3 to 5 inclusive and in which $n+n^1+2$ is not less than 11 and not more than 14 which comprises acylating the corresponding hydroxycycloalkanone with an acylating agent.

37. A process for the production of a compound of the formula



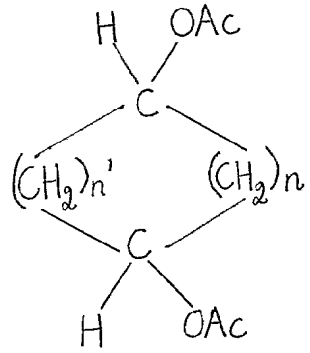
wherein Ac is the acyl radical of a hydrocarbon carboxylic containing from 1 to 12 carbon atoms inclusive, n is a whole number from 5 to 9 inclusive, n^1 is a whole number from 3 to 5 inclusive and in which $n+n^1+2$ is not less than 11 and not more than 14 which comprises reacting the corresponding cycloalkanediol with an acylating agent to obtain the corresponding cycloalkanediol diacylate.

38. A compound of the formula



wherein Ac is the acyl radical of a hydrocarbon carboxylic acid containing from 1 to 12 carbon atoms inclusive, n^1 is a whole number from 3 to 5 inclusive and in which $n+n^1+2$ is not less than 11 and not more than 14 when prepared by a process as claimed in claim 36.

39. A compound of the formula:



wherein Ac is the acyl radical of a hydrocarbon carboxylic acid containing from 1 to 12 carbon atoms inclusive, n is a whole number from 5 to 9 inclusive, n^1 is a whole number from 3 to 5 inclusive and in which $n + n^1 + 2$ is not less than 11 and not more than 14 when prepared by a process as claimed in claim 37.

40. A pharmaceutical composition comprising as the active ingredient a compound as claimed in any of claims 1 to 14 together with a pharmaceutically acceptable carrier.

41. A process for the preparation of a compound as claimed in any of claims 1 to 14 substantially as herein described with reference to any one of the Examples.

42. A compound as claimed in any of claims 1 to 14 when prepared by a process as claimed in any of claims 15 to 35 or 41.

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